Genomics

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Night-time predation by Steller sea lions

New insight into the feeding habits of these mammals will help conservation attempts.

Measures have been taken to curtail commercial fishing of walleye pollock (*Theragra chalcogramma*) in Alaska in an attempt to stop the decline of its endangered population of Steller sea lions (*Eumetopias jubatus*). But our night-time observations of these mammals in Prince William Sound using infrared scanning technology, combined with acoustic surveillance of their prey's behaviour, reveal that the sea lions feed exclusively on Pacific herring (*Clupea pallasi*), which are less abundant than pollock but are found closer to the surface at night.

Food limitation is the principal factor in the decline of Steller sea lion populations[1–4]. This decline could be explained by competition with commercial fisheries, as it has coincided with the growth of the pollock-fishing industry, which has become one of the largest fisheries in the world, or it could be related to a change in predator–prey relationships, possibly driven by ocean climate shifts[5]. Central to the uncertainty surrounding the drop in the numbers of Steller sea lions is a lack of observational data on their foraging ecology. There is no quantitative information available that directly relates the foraging behaviour of these animals to the abundance of prey species.

During the winter period, nutritional stress is high. Sonar surveys[6,7] of the abundance and distribution of adult Pacific herring and walleye pollock in winter have been made in Prince William Sound in Alaska since the early 1990s[8]. Steller sea lions were seen during the day near herring schools, but as no foraging activity was detectable, the significance of this co-occurrence was questionable.

We complemented our sonar surveys during March 2000 with infrared scanning of the Steller sea lions. This technology, which is widely used in night-time military operations and surveillance, enabled us to monitor the animals' activity during the hours of darkness. Our system had a $27° \times 18°$ field of view and a spectral response of 7–14 μm.

The estimated herring biomass in Prince William Sound in the sonar survey of March 2000 was 7,281 metric tonnes (95% confidence interval, 5,898–8,664). The estimate of pollock biomass was 28,277 metric tons (95% confidence interval, 26,034–30,420). Despite the much greater abundance of pollock, the infrared system revealed that foraging by Steller sea lions was exclusively on herring and was conducted only at night. Foraging activity was intense on dense herring schools (Fig. 1).

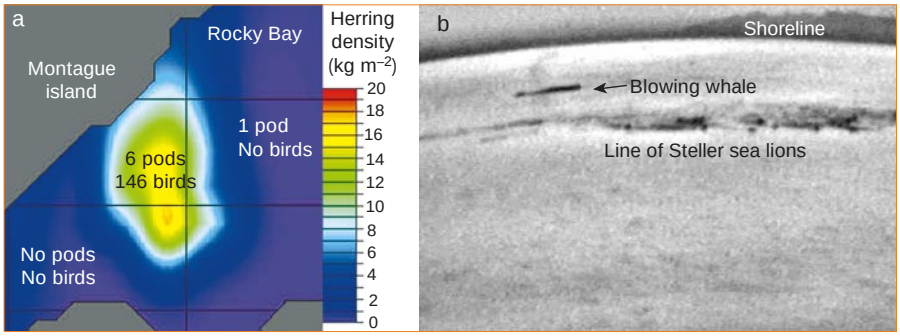

Figure 1 Location of groups (pods) of Steller sea lions around herring schools. **a,** Combined acoustic and infrared sensors reveal sea lions and birds located on the surface above the herring school at night in Rocky Bay, Prince William Sound (March 2000). **b,** Infrared video image showing a line of Steller sea lions and a humpback whale on the sea surface above a school of herring.

Steller sea lions were often observed swimming side by side in a row of 50 or more individuals along the edges of a school, suggesting that they were herding the herring. Humpback whales and seabirds were also seen to be feeding alongside the sea lions (Fig. 1). By contrast, no sea lions were coincident with pollock schools.

The sonar records revealed herring schools at depths of 10–35 m at night, but deeper during the day. Walleye pollock, on the other hand, remained at depths of over 100 m during both day and night. Pollock schools were also found in less protected regions and were further offshore. Although Steller sea lions are capable of dives exceeding 250 m (ref. 9), the more accessible distribution of herring at night may be the primary factor in the foraging behaviour of the sea lions. This distribution of herring is characteristic during an extended overwintering period in the North Gulf of Alaska.

Our results indicate that the dependence of Steller sea lions on herring as prey has been underestimated. The infrared scanning technology that has led us to this conclusion should also help in the evaluation of night-time foraging behaviour of other marine mammals and seabirds, with its remarkable ability to detect individual fish flipping on the sea surface at a distance of 5–30 m, as well as sea lions, whales and birds at over 100 m.

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Genomics

Genes lost during evolution

One of the main conclusions presented by the International Human Genome Sequencing Consortium is that "hundreds of genes appear to have resulted from horizontal gene transfer from bacteria at some point in the vertebrate lineage"[1]. We noticed that a significant proportion of these human genes have closely related orthologues in the primitive eukaryote *Dictyostelium*. This observation supports independent gene loss in multiple lineages (worm, fly, yeast, plants) rather than horizontal gene transfer from bacteria.

The human genome sequence revealed 113 genes that share a high degree of identity with bacterial genes, but are absent in the completely sequenced genomes of *Caenorhabditis elegans*, *Drosophila melanogaster*, *Saccharomyces cerevisiae* and *Arabidopsis thaliana*[1]. Do these genes represent examples of horizontal gene transfer from bacteria to the vertebrate lineage, or were they present in both prokaryotes and early eukaryotes, but subsequently lost from all non-vertebrate eukaryotic lineages? Although this latter possibility may seem unlikely, we recently identified a gene in *Dictyostelium* that is clearly an orthologue of the gene that encodes soluble

adenylyl cyclase in bacteria and vertebrates, but has not been identified in other eukaryotes[2]. *Dictyostelium* is located in the evolutionary tree between plants and the fungi/animal crown[3], and sequencing of its genome is approaching completion[4] (see also http://dictybase.org).

We used all 113 listed human genes to screen for homologous sequences in *Dictyostelium* (27 February 2001; see supplementary information). A TBLASTN screen of the *Dictyostelium* database yielded 36 sequences with expectation values of less than 10^{-10}. BLASTX analysis with the obtained *Dictyostelium* DNA sequences against GenBank identified 11 genes that represent clear *Dictyostelium* orthologues of human genes: the human sequences share a higher degree of identity with *Dictyostelium* than with bacterial sequences, and the bacterial sequences score more highly with respect to *Dictyostelium* than they do to humans (on the basis of BLAST expectation values). A further 17 *Dictyostelium* sequences share a high degree of identity with the human sequence, but are not obvious intermediates between the bacterial and vertebrate orthologues (see supplementary information). Thus, in at least 11 cases, the *Dictyostelium* and human genes have a common ancestor, eliminating the need to invoke horizontal gene transfer from bacteria.

One of the human proteins with an orthologue in *Dictyostelium* is monoamine oxidase (MAO). Phylogenetic analysis of this enzyme reveals a gene duplication late in the vertebrate lineage (MAO-A and MAO-B in Fig. 1). These paralogues seem to share a predecessor with *Dictyostelium*, indicating that monoamine oxidase was present in early eukaryotes, and implying that the gene has been lost in worm, fly, plants and yeast.

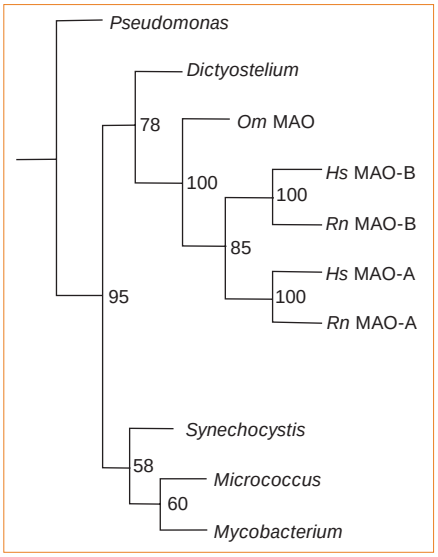

Figure 1 Phylogenetic analysis of monoamine oxidase (MAO). Numbers indicate values of bootstrap analysis ($n = 100$). Hs, *Homo sapiens*; Rn, *Rattus norvegicus* (rat); Om, *Oncorhynchus mykiss* (rainbow trout).

Within the group of 113 genes proposed to have entered the human genome by horizontal gene transfer from bacteria, we have identified at least 11 that probably arose through normal evolution with gene loss in several lineages, suggesting that gene loss is not a rare event. With several ongoing genomic sequencing projects for lower eukaryotes, it will be interesting to see how many genes have truly undergone horizontal transfer.

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Supplementary information is available at http://www.nature.com or as paper copy from the London editorial office of *Nature*.

Bone-marrow transplantation

Failure to correct murine muscular dystrophy

Bone-marrow cells have the potential to differentiate into other cell types such as muscle fibres, and can be transplanted into acutely[1] or chronically[2] damaged muscle as a way of delivering normal dystrophin (the protein that is defective or missing in Duchenne's muscular dystrophy) to the skeletal and heart muscle of *mdx* mice[2,3], an animal model for this disease. But the corrective potential of this approach has been hard to estimate against the high background of muscle fibres that spontaneously revert to synthesizing dystrophin, a feature of the original *mdx* mutation[4]. Here we test the long-term efficacy of bone-marrow transplantation in a different *mdx* mutant which is free of this problem and find that it has no impact on murine muscular dystrophy.

The *mdx4cv* mutant (in which a C-to-T nucleotide transition generates a stop codon in exon 53 of the dystrophin gene) has almost no background of revertant fibres in skeletal muscle[4]. We sublethally irradiated (900 cGy) a group of 15 8-week-old *mdx4cv* mice (C57Bl/6/Ly-5.2 background) and transplanted them with a total of 1.5×10^7 bone-marrow cells from a pool of 6-week-old, co-isogenic (C57Bl/6/Ly-5.1) animals. We killed the mice at regular intervals from 9 weeks to 10 months after transplantation, and monitored the engraftment of donor cells by cytofluorimetric analysis of the proportion of Ly-5.1 marker compared with Ly-5.2. The degree of chimaerism averaged $85 \pm 2.7\%$ in bone marrow (mean ± s.e.m.), $93 \pm 1.1\%$ in spleen, $92 \pm 2.9\%$ in thymus and $94 \pm 0.8\%$ in peripheral blood throughout the follow-up study.

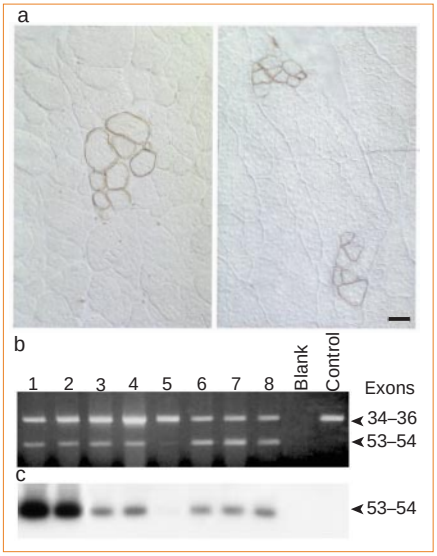

Figure 1 Expression of dystrophin in *mdx* mice 6 months after transplantation with bone-marrow cells from co-isogenic, normal donors. **a,** Immunohistochemical staining of frozen sections of tibialis muscle with an anti-dystrophin monoclonal antibody. Scale bar, 50 μm. **b,** Detection of wild-type dystrophin mRNA by RT-PCR amplification using specific primers for exons 53 and 54 in samples of total RNA extracted from skeletal muscle of transplanted *mdx* mice (lower bands). A fragment encompassing exons 33–36 in both wild-type and mutant dystrophin RNA is amplified as an internal control (upper bands). Lanes 1–8, samples from transplanted mice; 'blank', PCR assay without RNA; 'control', mock-transplanted *mdx* control. **c,** Southern-blot hybridization with an internal, specific oligonucleotide probe for exon 53.

We counted dystrophin-positive (dys^+) fibres in histological sections of representative muscles (tibialis anterior, quadriceps, diaphragm) after immunohistochemical staining with an anti-dystrophin antibody in transplanted and age-matched, mock-transplanted, control *mdx4cv* mice. Clusters of dys^+ fibres were apparent in muscle sections of transplanted animals, averaging $0.23 \pm 0.05\%$ (minimum, 0.06%; maximum, 0.54%) throughout the 10-month study (Fig. 1a). The proportion of dys^+ fibres in control animals averaged $0.14 \pm 0.03\%$ (minimum, 0.02%; maximum, 0.33%), a statistically significant difference ($F = 5.99$, $P = 0.02$). In neither group was there any significant increase in the number of dys^+ fibres in young (under 5 months) and old (over 12 months) animals. The average number of fibres contained in each dys^+ cluster varied from 3 to 30, with no significant change with age in either group.

To demonstrate the presence of normal dystrophin in the muscle of transplanted mice (the antibody does not distinguish between corrected and revertant fibres), we developed a polymerase chain reaction with reverse transcription (RT-PCR) assay to distinguish wild-type dystrophin messen-